

Differential Immune Responses to Primary Measles-Mumps-Rubella Vaccination in Israeli Children

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Measles remains an important cause of morbidity and mortality worldwide, primarily due to problems associated with delivery of the live attenuated vaccine to susceptible populations. In some developed countries, there is concern about the effects of immunization on the immune system. In this study, we analyzed the responses of 12-month-old Bedouin and Jewish children living in Israel to routine measles-mumps-rubella (MMR) vaccination. Seroconversion to measles was 99% in Bedouin and 79% in Jewish children ($P < 0.01$), and that to mumps and rubella was 92 to 100% in both groups. Measles neutralizing antibody titers were higher in Bedouin (333 ± 39 mIU/ml) than Jewish (122 ± 60 mIU/ml) children ($P < 0.002$). Immunoglobulin G levels were higher in Bedouin than Jewish children ($P = 0.007$) and increased after vaccination ($P = 0.0009$). Leukocyte ($P < 0.02$) and lymphocyte ($P = 0.04$) counts were higher and CD4 lymphocyte percentages were lower ($P < 0.001$) in Bedouin than Jewish children before and after vaccination. Leukocyte counts and natural killer cell numbers did not change after vaccination, but lytic activity increased in Bedouin children ($P < 0.005$). Spontaneous proliferation of cultured peripheral blood mononuclear cells increased with vaccination, but there were no changes in the proliferative responses to phytohemagglutinin or tetanus toxoid. In summary, no adverse effects of MMR vaccination on immune function were detected. However, there were differences in underlying immunologic parameters and in response to the measles component of the vaccine between Bedouin and Jewish children. It is not known whether genetic differences or environmental exposure accounts for these differences.

Measles remains a major worldwide public health problem, with approximately 800,000 deaths per year, despite the existence of a safe and effective live virus vaccine (1). Most of the cases and deaths occur in the developing world, but outbreaks associated with low vaccine coverage continue to occur in developed countries as well (1, 20). Although rates of seroconversion are dependent on the age of vaccination (6, 17, 27), it is generally presumed that responses to measles vaccination are otherwise similar in various populations and that the primary problem of measles control lies in vaccine delivery. Seroconversion to the measles component of measles-mumps-rubella (MMR) vaccine given at 12 months is generally 90 to 98%, but studies have suggested substantially lower rates in some populations (3, 17, 22). It is not known whether this variability is determined by the quality of the vaccine at the time it is delivered or by differences in host responses. Both genetic and environmental factors could influence response rate.

The Negev region of Israel provides a unique opportunity to study the immune responses to measles vaccination in relatively homogeneous populations with different ethnic backgrounds and living conditions. Bedouin Arabs have a high birth rate, with an average of nine children in each family, live in relatively crowded conditions, and suffer from a substantial

burden of infectious diseases. The birth rate is lower in Jewish families (2.7 children/family), living conditions are generally less crowded, and the burden of infectious diseases is less. Both populations have similar access to modern health care services. The current study evaluated immune responses to primary MMR vaccination at 12 months as well as the generalized effects of primary MMR vaccination on immune function in Bedouin and Jewish Israeli children.

MATERIALS AND METHODS

Study population. The study population consisted of 104 healthy infants receiving their primary MMR vaccination at 12 months of age, administered according to the guidelines of the Israeli Ministry of Health as part of the routine national immunization program. Children were enrolled in 1995, and all were receiving their immunizations at Preventive Maternal and Child Health Centers administered by the Ministry of Health. The vaccine administered was MMRII (Merck, West Point, Pa.), consisting of the Moraten strain of measles virus (MV), the Jeryl Lynn B strain of mumps virus, and the Wistar RA 27/3 strain of rubella virus. Vaccines administered at the same visit were diphtheria, pertussis (whole cell), tetanus (DPT), and oral polio vaccine. The study was approved by the Soroka Medical Center Committee for the Protection of Human Subjects, and the parents or guardians of all participants gave written informed consent for the study.

Ninety-one infants with adequate samples included 77 Bedouin Arab infants (59% males) and 14 Jewish infants (71% males). All infants had blood drawn immediately before receiving MMR and were asked to return for repeat venipuncture 30 days later. The dates of the follow-up samples ranged from 28 to 111 days after the administration of MMR vaccine. There were no cases of measles reported in the Negev region from the time of birth of the study infants until the time of the second venipuncture.

Collection of blood samples. Blood (5 to 6 ml), collected by venipuncture, was placed into a 15-ml tube containing 50 IU of heparin and immediately transferred to the research laboratory, where total blood cell counts and flow cytometry assays were performed and mononuclear cells were separated. Assays re-

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quiring live cells were run within 4 h. Plasma samples were stored in aliquots at -20°C for up to 1 year until tested. Because of small and variable sample volumes, not all assays could be run on all samples. Priority was given to antibody assays and to leukocyte (WBC) counts.

Total WBC counts. One milliliter of blood was used for WBC counts, which were performed with a Coulter counter (CELL DYN 1600 system; Abbott Laboratories, Diagnostic Division, Abbott Park, Ill.) following the manufacturer's protocol.

Cell cultures. Monolayer cultures of Vero cells, grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS) (Maagar Biological Industries, Beit Haemek, Israel), glutamine, and antibiotics, were used for the growth of measles virus stocks and for the plaque reduction neutralization (PRN) assay. K562 erythroleukemia cells, grown in RPMI 1640 medium (Maagar) supplemented with 10% FCS, glutamine, and antibiotics, were used as target cells for the measurement of natural killer (NK) cell cytotoxic activity.

MV neutralizing antibody. Paired sera from before and after vaccination were run in parallel and tested for MV neutralizing antibody with a modified 50% PRN assay (25). Briefly, serial twofold dilutions (1:4 to 1:4,096) of heat-inactivated sera (56°C for 30 min) were prepared in 96-well flat-bottom microtiter plates. Equal volumes of the Edmonston strain of MV containing 50 to 100 PFU/ml were added to duplicate wells of each dilution. The mixtures were incubated for 1 h at room temperature, and then 5×10^4 Vero cells were added to the serum-virus mixtures. After 5 days of incubation in a humidified atmosphere at 37°C in 5% CO_2 , plaques were counted. A reference serum calibrated against an international reference serum (5) was included in each run. PRN titers were expressed as the reciprocal of the highest dilution that gave 50% plaque reduction and adjusted to the international standard. A fourfold rise in titer was considered seroconversion.

Virus-specific ELISA-reactive IgG antibody. Mumps and rubella virus-specific immunoglobulin G (IgG) antibodies were assayed with commercial enzyme-linked immunosorbent assay (ELISA) kits (Human mbH, Aachen, Germany) according to the manufacturer's instructions. Seroconversion was defined as a change from negative to positive as defined by the manufacturer.

Ig levels. Total IgM and IgG levels in sera were determined in the clinical immunology laboratory of Soroka Medical Center, using the Beckman immunochemistry system according to the manufacturer's instructions (Beckman Instruments, Inc., Galway, Ireland). Total IgE levels were determined by the IMX system (Abbott).

Lymphoproliferation assays. Peripheral blood mononuclear cells (PBMCs) were separated from heparinized blood by centrifugation on Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N.J.) density gradients. PBMCs were washed in phosphate-buffered saline (PBS) and resuspended at $10^6/\text{ml}$ in RPMI 1640 supplemented with 5% heat-inactivated FCS, 10 mM HEPES (Gibco BRL), and antibiotics. Spontaneous proliferation and proliferative responses to phytohemagglutinin (PHA; Wellcome HA15) and preservative-free tetanus toxoid (TT), a gift from S. Udem (Lederle Labs) were tested on fresh cells. Spontaneous proliferative activity was measured by culturing 2×10^5 (200 μl) unstimulated PBMCs in triplicate in flat-bottom 96-well microtiter plates (Corning, Inc.) at 37°C in 5% CO_2 for 1, 3, and 6 days. Data are expressed as cpm incorporated into the cells. Proliferative activity of lymphocytes to PHA or TT was determined after culturing the cells with 2.5- $\mu\text{g}/\text{ml}$ PHA for 3 days or with 1:10,000 TT for 6 days. The samples were pulsed with 1 μCi of [*methyl*- ^3H]thymidine (5,000 mCi/mmol; NRCN, Beer-Sheva, Israel) 18 h prior to harvesting. Data are expressed as cpm incorporated into PHA- or TT-stimulated cells – cpm of unstimulated cells (Δcpm).

Flow cytometry. Whole-blood samples (150 μl) were reacted with 20 μl of anti-CD4/CD8 or anti-CD56 antibodies (Becton Dickinson, Mountain View, Calif.) according to the manufacturer's instructions and incubated for 30 min on ice in the dark. To remove erythrocytes, 1.4 μl of fluorescence-activated cell sorter (FACS) lysing solution was added to each tube and mixed gently. After incubation (10 min, room temperature, in the dark), samples were pelleted ($300 \times g$, 7 min, room temperature) and supernatant fluids were aspirated, leaving approximately 50 μl of fluid. Cell pellets were gently resuspended and washed twice in f-PBS (Becton Dickinson). After final resuspension, 800 μl of FACS storing solution was added, and samples were analyzed immediately or stored up to 14 days at 4°C in the dark. Before the analysis, samples were gently mixed and filtered through silk filters. FACS analysis was done with a FACStar Plus flow cytometer (Becton Dickinson). Data are reported as percentage of lymphocytes positive.

NK cell lysis. NK cytotoxic activity was tested in a ^{51}Cr -release assay by incubating lymphocyte effector cells at ratios of 1:100, 1:50, and 1:25 in 96-well U-bottom microtiter plates (Corning) at 37°C with 2×10^4 ^{51}Cr -labeled K562 erythroleukemia target cells. After 4 h of incubation, the plates were centrifuged

TABLE 1. Seroconversion rates for Jewish and Bedouin infants to measles, mumps, and rubella viruses after MMR vaccination at 12 months of age

Virus	Test	No. positive/total (%)		<i>P</i>
		Jewish	Bedouin	
Measles	PRN	11/14 (79)	76/77 (99)	<.01
Mumps	EIA ^a	11/11 (100)	61/65 (94)	NS ^b
Rubella	EIA	10/10 (100)	60/65 (92)	NS

^a EIA, enzyme immunoassay.

^b NS, not significant.

for 10 min at $300 \times g$, and 0.1 ml of supernatant fluid was harvested from each well for determination of ^{51}Cr release. For maximal ^{51}Cr release, 0.1 of 2 N HCl was added to target cells. The percentage of specific lysis was calculated according to the formula % specific lysis = [(test cpm – spontaneous cpm)/(maximum lysis cpm – spontaneous cpm)] $\times 100$.

Spontaneous release did not exceed 20%. Results are expressed as mean percent specific ^{51}Cr release.

Statistical analysis. Statistical analysis was performed with SPSS or Statview software. Wilcoxon's signed-rank test or Student's paired *t* test was used to estimate the significance of the differences between post- and preimmunization values. Comparison between groups was performed with the Mann-Whitney U, Student's *t*, or Fisher's exact test, as indicated. Quantitative data are reported \pm standard error.

RESULTS

Virus-specific antibody responses. Specific measles virus neutralizing antibody and mumps and rubella virus IgG ELISA antibodies were determined before and after primary MMR vaccination in paired sera. Overall seroconversion was 96% for measles, 95% for mumps, and 93% for rubella. Seroconversion was similar in Jewish and Bedouin infants for mumps and rubella, but differed for measles (Table 1). Measles seroconversion was 79% for Jewish infants and 99% for Bedouin infants ($P < 0.01$; Fisher's exact analysis). In general, nonresponders were different for each component of the vaccine.

Failure to respond to measles was not due to differences in the levels of maternal antibody to this virus. Eleven of the Bedouin infants (14%) and 3 of the Jewish infants (21%) had evidence of persistent maternal antibody to MV, as measured by preimmunization titers ranging from 1:16 to 1:64 ($P > 0.2$). All of these infants with residual maternal antibody to MV responded to the measles component of the vaccine. Within each ethnic group, infants with or without residual maternal antibody were combined for further analysis.

To determine whether the antiviral titers achieved after vaccination were similar in the Jewish and Bedouin infants, titers were compared for measles and mumps, the two viruses for which the tests used provide quantitation of the amount of antibody (Fig. 1). Measles neutralizing antibody titers after vaccination were lower in Jewish children (122 ± 60 mIU/ml) than in Bedouin children (333 ± 39 mIU/ml) ($P = 0.0016$; Student's *t* test on log-transformed data), and this was primarily due to differences in the children tested more than 6 weeks after immunization. If only the titers of seroconverters are compared the difference remains, but is no longer statistically significant (240 ± 55 , Jewish; 347 ± 38 , Bedouin; $P = 0.19$). Mumps antibody titers were not different between the groups.

Effect of immunization on levels of total IgM, IgG, and IgE in serum. To identify generalized effects of the MMR vaccine on immune responses, total amounts of IgM, IgG, and IgE

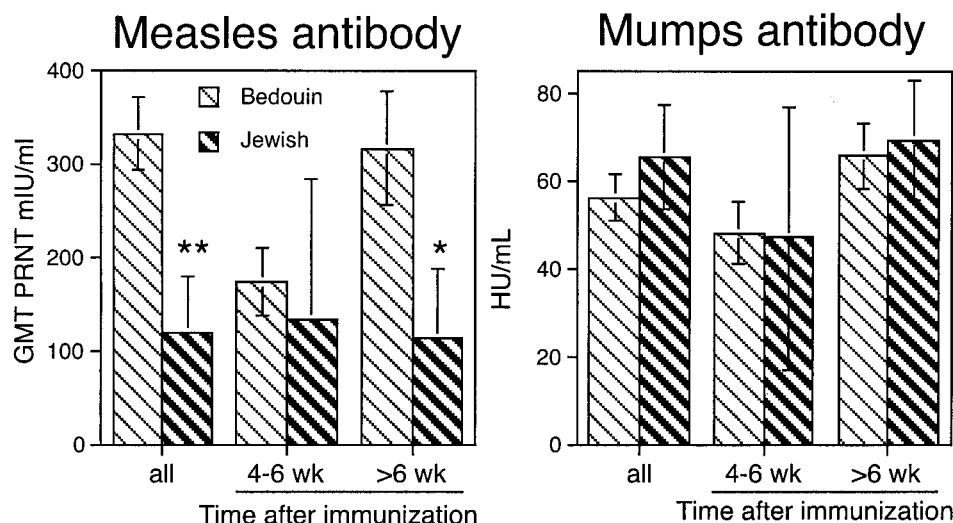


FIG. 1. Levels of antibody to measles and mumps viruses after vaccination. Antibody was measured in plasma by plaque reduction neutralization (PRNT) for measles virus and by quantitative enzyme immunoassay for mumps. Measles antibody data were log transformed for analysis. Data are shown for all Bedouin ($n = 77$) and Jewish ($n = 14$) children and stratified by time after vaccination for those for whom this information was available. Jewish children had lower levels of antibody to measles than did Bedouin children. HU, units as defined by the manufacturer. *, $P < 0.05$; **, $P < 0.01$ (Student's t test).

were measured (Fig. 2). Total serum IgM levels prior to immunization ranged from 25 to 200 mg%, IgG levels ranged from 498 to 1,770 mg%, and IgE levels ranged from 1 to 866 IU. There were no significant differences between the Jewish and Bedouin infants in IgM or IgE levels either before or after vaccination. However, the Bedouin infants had higher levels of IgG before (994 ± 261 versus 765 ± 264 mg%; $P = 0.0069$) and after ($1,081 \pm 270$ versus 842 ± 285 mg%; $P = 0.0068$; Student's t test) vaccination and had a significant increase in IgG level after vaccination ($P = 0.0009$; Wilcoxon's signed-rank test).

Effect of immunization on WBC counts. Measles is associated with a decrease in lymphocyte count and in numbers of both $CD4^+$ and $CD8^+$ T cells (26), and revaccination with MMR is associated with a decrease in WBC count (24, 32). To

investigate the effect of primary vaccination on WBC numbers, WBCs, lymphocytes, and $CD4^+$ and $CD8^+$ subpopulations of T lymphocytes and their ratios were assessed (Fig. 3). WBC and lymphocyte counts did not change after immunization in either group, but differed between the groups. Bedouin children had higher WBCs than Jewish children both before ($13,010 \pm 603$ versus $10,389 \pm 582$ cells/mm³; $P = 0.0186$; Mann-Whitney U) and after ($12,640 \pm 652$ versus $9,455 \pm 756$ cells/mm³; $P = 0.0054$) vaccination. After vaccination, lymphocyte counts of Bedouin children were higher ($8,110 \pm 852$ cells/mm³) than those of Jewish children ($6,115 \pm 582$; $P = 0.0412$; Mann-Whitney U). Bedouin children had lower percentages of $CD4^+$ T cells at both time points ($24.6\% \pm 1.03\%$ before, $23.3\% \pm 1.13\%$ after) than Jewish children ($34.5\% \pm 2.02\%$ before, $P = 0.0006$; $32.2\% \pm 1.77\%$ after, $P = 0.0004$;

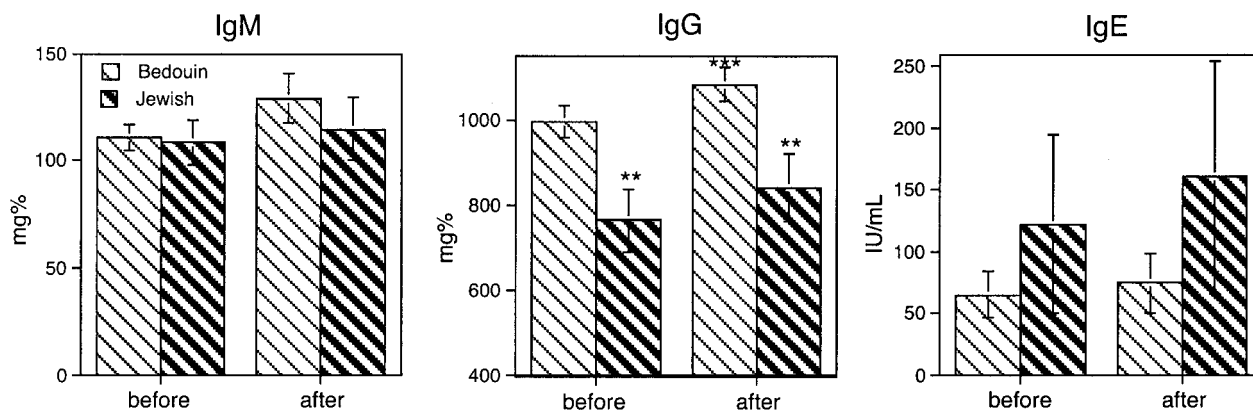


FIG. 2. Levels of Ig in Bedouin and Jewish children before and after vaccination. Total levels of IgM, IgG, and IgE were measured on plasma from Bedouin ($n = 46$ to 48) and Jewish ($n = 13$) children before and after vaccination. Levels of IgM and IgE were similar between groups and did not change after immunization. Levels of IgG were higher in Bedouin children than Jewish children at both time points and increased after vaccination. **, $P < 0.01$ comparing Bedouin and Jewish children (Student's t test); ***, $P < 0.001$ comparing levels before and after vaccination (Wilcoxon's signed-rank test).

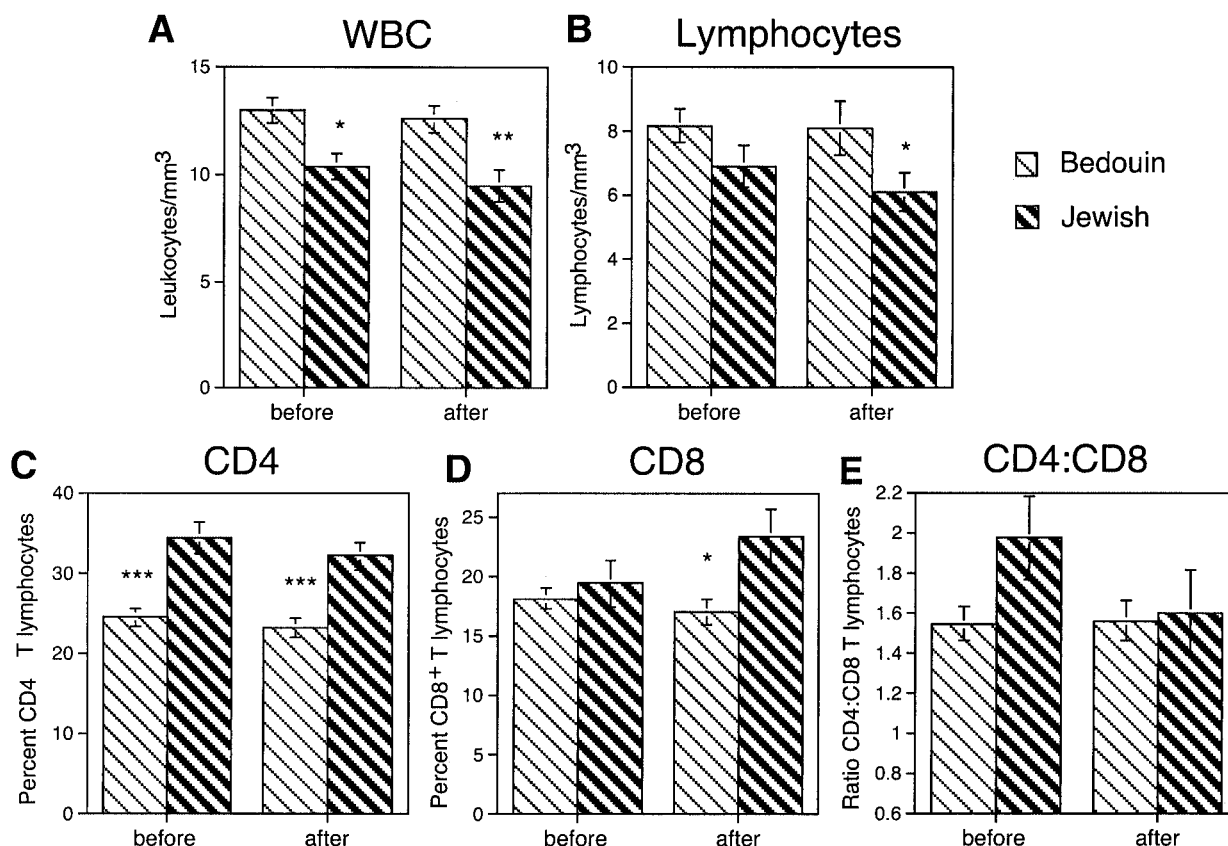


FIG. 3. WBC counts in Bedouin and Jewish children before and after vaccination. Numbers of WBCs and lymphocytes and percentages of CD4⁺ and CD8⁺ T lymphocytes were measured before and after vaccination in Bedouin ($n = 25$ to 75) and Jewish ($n = 9$ to 13) children. There were no changes in these parameters. WBCs were higher in Bedouin children than Jewish children before and after vaccination. Lymphocyte counts were higher and the percentages of CD4⁺ and CD8⁺ T lymphocytes were lower in Bedouin children than Jewish children. CD4/CD8 ratios were not different. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (Mann-Whitney U test for comparison between groups and Wilcoxon's signed-rank test for comparison of values before and after vaccination).

Mann-Whitney U). Percentages of CD8⁺ cells were not different between the groups prior to vaccination, but Jewish children had a higher percentage of CD8⁺ T cells after vaccination ($23.4\% \pm 2.36\%$) than Bedouin children ($17.1\% \pm 1.06\%$; $P = 0.0153$; Mann-Whitney U). This resulted in a higher CD4/CD8 ratio for Jewish children (1.98 ± 0.21) than Bedouin children (1.55 ± 0.09) ($P = 0.0583$) prior to vaccination, but no difference after vaccination, as the ratio decreased for Jewish children (1.61 ± 0.21).

Lymphocyte proliferative responses. A hallmark of immunologic abnormalities occurring during MV infection is the suppression of in vivo and in vitro cellular immune responses (7). To investigate the effect of vaccination on these responses, lymphocyte proliferation assays were performed (Table 2). There were no differences between the responses of Bedouin and Jewish children. Spontaneous proliferation, indicative of in vivo lymphocyte activation, was measured after 24 h of incubation and showed an increase both in children studied at 4 to 6 weeks and more than 6 weeks after immunization. Responses to stimulation with PHA and TT were unchanged after immunization.

NK cells. Changes in NK cells were assessed by determining the percentage of CD56⁺ lymphocytes and the lytic activity of PBMCs for NK target cells (Fig. 4). There was no change in the

percentage of CD56⁺ cells after immunization for either group. Cytotoxic activity increased for Bedouin children after vaccination from $20\% \pm 14.2\%$ to $33.6\% \pm 3.1\%$ specific lysis ($P = 0.0044$; Wilcoxon's signed-rank test), but did not change for Jewish children.

TABLE 2. In vitro proliferation of PBMCs before and after vaccination^a

Stimulation	Time (wk)	<i>n</i>	Proliferation (cpm)		<i>P</i> ^b
			Before	After	
None (dl) (day 1)	4-6	20	837 ± 120	1,166 ± 188	0.0401
	>6	20	573 ± 140	639 ± 183	0.0052
PHA	4-6	21	29,294 ± 2,582	34,829 ± 2,635	0.085
	>6	20	28,077 ± 7,271	30,025 ± 5,109	NS ^c
TT	4-6	16	310 ± 125	399 ± 208	NS
	>6	21	881 ± 348	1,018 ± 205	NS

^a Data are combined for all children. There were no differences between Jewish and Bedouin children for these parameters.

^b Wilcoxon's signed-rank test.

^c NS, not significant.

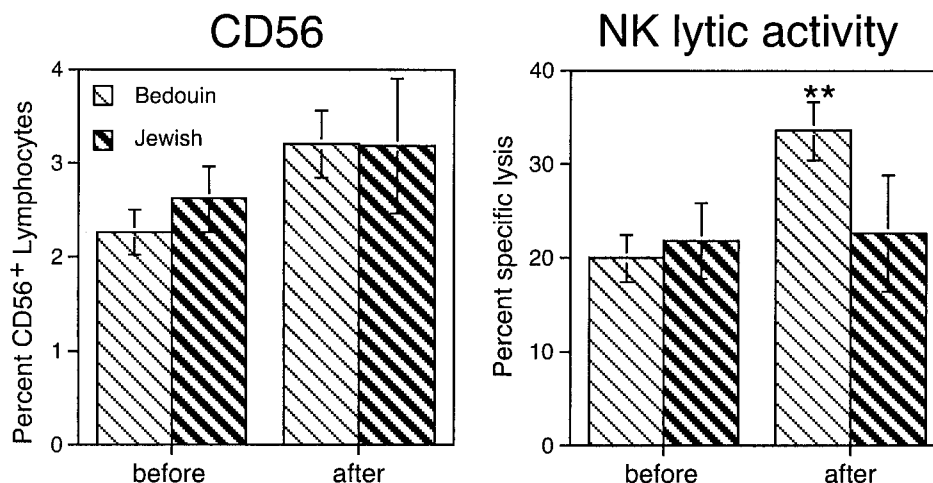


FIG. 4. NK cell numbers and lytic activity in Bedouin and Jewish children before and after vaccination. Numbers of NK cells were determined by quantitating the percentage of lymphocytes that were CD56⁺ in Bedouin ($n = 31$ to 41) and Jewish ($n = 11$ to 13) children before and after vaccination. No differences were detected. Activity of NK cells was measured by lysis of K562 cells. There were no differences between Bedouin and Jewish children, but NK activity increased after vaccination for Bedouin children. **, $P < 0.01$; Wilcoxon's signed-rank test.

DISCUSSION

This study of immune responses after routine MMR immunization of 1-year-old infants in Israel has detected no adverse effects on in vitro measures of immune function after vaccination. Lymphoproliferation in response to mitogens and antigens, WBC counts, and NK activity were unchanged or improved after vaccination. However, unexpected differences in the immune status and responses of Bedouin and Jewish infants receiving care in the same system were observed. At the time of vaccination at 12 months of age, Bedouin children had higher WBCs, higher levels of IgG, and a lower percentage of CD4⁺ T cells than Jewish children and responded better to the measles component of the vaccine.

MV is a highly immunosuppressive virus, and most of the morbidity and mortality is caused by increased susceptibility to other infectious diseases (7). Documented immunologic abnormalities include leukopenia, loss of delayed-type hypersensitivity skin test responses to recall antigens, suppressed in vitro lymphoproliferative responses to mitogens and recall antigens, and generalized immune activation with skewing of the immune response toward type 2 cytokine production (7, 9, 10, 16). In addition, measles leads to an increased production of IgE and increased susceptibility to the autoimmune disease acute disseminated encephalomyelitis (8, 15). The standard-dose live attenuated measles vaccine virus is safe and generally free of significant side effects, but experience with adverse effects associated with the use of the high-titer vaccine in infants (12, 18) and transient abnormalities in in vitro proliferative responses after primary vaccination of infants (13) have encouraged further study of the effects of vaccination on immune function of infants.

Lymphopenia is common during measles (26) and was observed after primary vaccination with the earliest versions of the measles vaccine (Edmonston B), which frequently induced a fever and rash (2). Also, a decrease in WBCs was observed in Canadian and Israeli children 1 month after revaccination with MMR (24, 32). However, WBCs were unchanged 2 and 12 weeks after primary vaccination of 6- to 9-month-old South

African infants with the Edmonston-Zagreb or Schwarz strains of measles vaccine (13) or after primary vaccination of 1- to 8-year-old children with MMR (19). We found no effect of primary vaccination on WBCs, lymphocyte percentages, or CD4/CD8 ratios after primary vaccination of 1-year-old infants with MMR, further suggesting that primary vaccination with the current vaccine has little effect on these parameters.

Despite the fact that leukocyte counts do not change after primary vaccination, lymphocyte function has been noted to change. In our study, increased spontaneous proliferation of cultured PBMCs indicated immune activation and correlates with previous observations of higher expression of activation markers on lymphocytes (27) and increases in β -2 microglobulin, neopterin, and soluble CD8 in plasma after measles vaccination (13). A number of studies have also reported altered T-cell function after primary measles vaccination. For instance, in Bangladeshi infants, *Candida* delayed-type hypersensitivity was reduced after measles vaccination (27) and many, but not all, studies have reported decreased in vitro lymphoproliferative responses to PHA, concanavalin A, TT, or purified protein derivative (13) after measles or MMR primary immunization (19, 21, 29). In the present study, proliferative responses to PHA and TT were unchanged. The differences between these various studies may reflect differences in the age of vaccination, time of analysis after vaccination, or populations studied.

The effect of vaccination on NK cells and their function has received limited attention. During wild-type MV infection, NK cell lytic activity is suppressed and type 1 interferon, an important activator of NK cell activity, is not detectable (11, 28). In this study, we have shown that NK cell numbers and function are preserved after vaccination. In Bedouin children, as in a previous study of Canadian children (21), there was an increase in NK cells or activity.

The differences between Bedouin and Jewish infants were unexpected. The low rate of seroconversion to measles is consistent with a previous study of Jewish school children in the same region in which 24% of 6-year-old children with a documented history of receiving MMR were seronegative (24).

The differences in seroconversion, as well as in IgG levels and WBC counts, could reflect genetic or environmental influences. A few previous studies have shown differences in serologic responses to measles vaccine in children of different ethnic backgrounds. Native Canadians have a higher rate of seropositivity (83%) than Caucasian Canadians (76%) and higher mean antibody levels (22). A lower proportion of African children seroconverted after receiving an aerosol-administered vaccine in South Africa than Indian children (4). Furthermore, twin studies of measles antibody levels suggest a role for genetic influences in the variation of responses to measles immunization (31). In American children, HLA class I and class II alleles have been linked to measles vaccine responsiveness. HLA class I alleles B8, B13, and B44 (14) and class II alleles DRB1*03 and DQA1*0201 (23) and HLA homozygosity (30) are all associated with measles seronegativity. Further study in larger numbers of individuals will be needed to determine the factors leading to differences in immune parameters and seroconversion to measles in Bedouin and Jewish children.

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REFERENCES

1. Anonymous. 2003. Update: global measles control and mortality reduction—worldwide, 1991–2001. *Morb. Mortal. Wkly. Rep.* **52**:471–475.
2. Black, F. L., and S. R. Sheridan. 1967. Blood leukocyte response to live measles vaccine. *Am. J. Dis. Child.* **113**:301–304.
3. Ceyhan, M., G. Kanra, G. Erdem, and B. Kanra. 2001. Immunogenicity and efficacy of one dose measles-mumps-rubella (MMR) vaccine at twelve months of age as compared to monovalent measles vaccination at nine months followed by MMR revaccination at fifteen months of age. *Vaccine* **19**:4473–4478.
4. Dilraj, A., F. T. Cutts, J. F. de Castro, J. G. Wheeler, D. Brown, C. Roth, H. M. Coovadia, and J. V. Bennett. 2000. Response to different measles vaccine strains given by aerosol and subcutaneous routes to schoolchildren: a randomised trial. *Lancet* **355**:798–803.
5. Forsey, T., A. Heath, and P. Minor. 1991. The 1st International Standard for anti-measles serum. *Biologicals* **19**:237–241.
6. Gans, H. A., A. M. Arvin, J. Galinus, L. Logan, R. DeHovitz, and Y. Maldonado. 1998. Deficiency of the humoral immune response to measles vaccine in infants immunized at age 6 months. *JAMA* **280**:527–532.
7. Griffin, D. E. 1995. Immune responses during measles virus infection. *Curr. Top. Microbiol. Immunol.* **191**:117–134.
8. Griffin, D. E., S. J. Cooper, R. L. Hirsch, R. T. Johnson, I. Lindo de Soriano, S. Roedenbeck, and A. Vaisberg. 1985. Changes in plasma IgE levels during complicated and uncomplicated measles virus infections. *J. Allergy Clin. Immunol.* **76**:206–213.
9. Griffin, D. E., and B. J. Ward. 1993. Differential CD4 T cell activation in measles. *J. Infect. Dis.* **168**:275–281.
10. Griffin, D. E., B. J. Ward, E. Jauregui, R. T. Johnson, and A. Vaisberg. 1989. Immune activation during measles. *N. Engl. J. Med.* **320**:1667–1672.
11. Griffin, D. E., B. J. Ward, E. Jauregui, R. T. Johnson, and A. Vaisberg. 1990. Natural killer cell activity during measles. *Clin. Exp. Immunol.* **81**:218–224.
12. Holt, E. A., L. H. Moulton, G. K. Siberry, and N. A. Halsey. 1993. Differential mortality by measles vaccine titer and sex. *J. Infect. Dis.* **168**:1087–1096.
13. Hussey, G. D., E. A. Goddard, J. Hughes, J. J. Ryon, M. Kerran, E. Carelse, P. M. Strebel, L. E. Markowitz, J. Moodie, P. Barron, Z. Latief, R. Sayed, D. Beatty, and D. E. Griffin. 1996. The effect of Edmonston-Zagreb and Schwarz measles vaccines on immune responses in infants. *J. Infect. Dis.* **173**:1320–1326.
14. Jacobson, R. M., G. A. Poland, R. A. Vierkant, V. S. Pankratz, D. J. Schaid, S. J. Jacobsen, J. St Sauver, and S. B. Moore. 2003. The association of class I HLA alleles and antibody levels after a single dose of measles vaccine. *Hum. Immunol.* **64**:103–109.
15. Johnson, R. T., D. E. Griffin, R. L. Hirsch, J. S. Wolinsky, S. Roedenbeck, I. Lindo de Soriano, and A. Vaisberg. 1984. Measles encephalomyelitis—clinical and immunologic studies. *N. Engl. J. Med.* **310**:137–141.
16. Kiepele, P., H. M. Coovadia, and P. Coward. 1987. T helper cell defect related to severity in measles. *Scand. J. Infect. Dis.* **19**:185–192.
17. Klinge, J., S. Lugauer, K. Korn, U. Heininger, and K. Stehr. 2000. Comparison of immunogenicity and reactogenicity of a measles, mumps and rubella (MMR) vaccine in German children vaccinated at 9–11, 12–14 or 15–17 months of age. *Vaccine* **18**:3134–3140.
18. Knudsen, K. M., P. Aaby, H. Whittle, M. Rowe, B. Samb, F. Simondon, J. Sterne, and P. Fine. 1996. Child mortality following standard, medium or high titre measles immunization in West Africa. *Int. J. Epidemiol.* **25**:665–673.
19. Munyer, T. P., R. J. Mangi, T. Dolan, and F. S. Kantor. 1975. Depressed lymphocyte function after measles-mumps-rubella vaccination. *J. Infect. Dis.* **132**:75–78.
20. Muscat, M., S. Glismann, and H. Bang. 2003. Measles in Europe in 2001–2002. *Eur. Surveill.* **8**:123–129.
21. Pabst, H. F., D. W. Spady, M. M. Carson, H. T. Stelfox, J. A. Beeler, and M. P. Krezolek. 1997. Kinetics of immunologic responses after primary MMR vaccination. *Vaccine* **15**:10–14.
22. Poland, G. A., R. M. Jacobson, S. A. Colbourne, A. M. Thampy, J. J. Lipsky, P. C. Wollan, P. Roberts, and S. J. Jacobsen. 1999. Measles antibody seroprevalence rates among immunized Inuit, Innu and Caucasian subjects. *Vaccine* **17**:1525–1531.
23. Poland, G. A., I. G. Ovsyannikova, R. M. Jacobson, R. A. Vierkant, S. J. Jacobsen, V. S. Pankratz, and D. J. Schaid. 2001. Identification of an association between HLA class II alleles and low antibody levels after measles immunization. *Vaccine* **20**:430–438.
24. Rager-Zisman, B., E. Bazarsky, A. Skibin, S. Chamney, I. Belmaker, I. Shai, E. Kordysh, and D. E. Griffin. 2003. The effect of measles-mumps-rubella (MMR) immunization on the immune responses of previously immunized primary school children. *Vaccine* **21**:2580–2588.
25. Rager-Zisman, B., and T. Merigan. 1973. A useful quantitative semimicro-method for viral plaque assay. *Proc. Soc. Exp. Biol. Med.* **142**:1174–1179.
26. Ryon, J., W. Moss, M. Monze, and D. E. Griffin. 2002. Functional and phenotypic changes in circulating lymphocytes from hospitalized Zambian children with measles. *Clin. Diagn. Lab. Immunol.* **9**:994–1003.
27. Schnorr, J. J., F. T. Cutts, J. G. Wheeler, S. M. Akramuzzaman, M. S. Alam, T. Azim, S. Schneider-Schaulies, and V. ter Meulen. 2001. Immune modulation after measles vaccination of 6–9 months old Bangladeshi infants. *Vaccine* **19**:1503–1510.
28. Shiozawa, S., N. Yoshikawa, K. Iijima, and K. Negishi. 1988. A sensitive radioimmunoassay for circulating alpha-interferon in the plasma of healthy children and patients with measles virus infection. *Clin. Exp. Immunol.* **73**:366–369.
29. Smedman, L., A. Joki, A. P. daSilva, M. Troye-Blomberg, B. Aronsson, and P. Perlmann. 1994. Immunosuppression after measles vaccination. *Acta Paediatr.* **83**:164–168.
30. St Sauver, J. L., I. G. Ovsyannikova, R. M. Jacobson, S. J. Jacobsen, R. A. Vierkant, D. J. Schaid, V. S. Pankratz, E. M. Green, and G. A. Poland. 2002. Associations between human leukocyte antigen homozygosity and antibody levels to measles vaccine. *J. Infect. Dis.* **185**:1545–1549.
31. Tan, P. L., R. M. Jacobson, G. A. Poland, S. J. Jacobsen, and V. S. Pankratz. 2001. Twin studies of immunogenicity—determining the genetic contribution to vaccine failure. *Vaccine* **19**:2434–2439.
32. Ward, B. J., N. Boulianne, S. Ratnam, M.-C. Guiot, M. Couillard, and G. DeSerres. 1995. Cellular immunity in measles vaccine failure: demonstration of measles antigen-specific lymphoproliferative responses despite limited serum antibody production after revaccination. *J. Infect. Dis.* **172**:1591–1595.